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GENETIC RELATIONSHIP ANALYSIS OF PLANTS IN THE GENUS *JATROPHA* IN THAILAND USING ISSR TECHNIQUES

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ABSTRACT

There are 5 species of Jatropha in Thailand. However, Jatropha curcas is the only one which is attracted as an alternative to biodiesel. It is widely distributed in many areas in Thailand. Therefore, objectives of this study were to determine the genetic relationships among 24 samples of the genus Jatropha using Inter Simple Sequence Repeats (ISSR). A total of 39 ISSR primers were used for initial screening, only 5 primers, MAO UBC808 UBC827 UBC873 and UBC835 were found to give polymorphic patterns. These ISSR primers amplified 54 polymorphic bands (0.35-2.3 Kb). The similarity coefficient using NTSYS pc (version 2.20e) ranged from 0.66-1.00 among J. curcas and interspecific level of Jatropha ranged from 0.472- 0.717. The dendogram was constructed base on SHAH clustering technique of UPGMA which divided the 24 samples into 3 groups. Group I consisted of 19 samples of J. curcas and J. multifida which J. curcas (Lampoon, Thailand) was closely related to J. curcas (Laos) with high similarity index was 1.000. Group 2 was J. integerrima (1), J. integerrima (2) and J. gossypifolia formed a group together. Within this group J. integerrima (1) and J. integerrima (2) were closer to each other with high similarity index was 0.943 and group 3 was J. podagrica.

KEYWORDS: Genetic Relationship, Jatropha, ISSR Technique

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Original Article

INTRODUCTION

Jatropha belonging to the family Euphobiaceae has around 200 species distributed throughout the tropical and subtropical regions of the world. *Jatropha* is a drought-resistant perennial crop able to grow in a wide range of soils, grows relatively quickly and producing seeds for 50 years. *Jatropha curcas* is the only one which is attracted as an alternative to biodiesel and it is a native plant of Mexico and Central America region. It has a very high oil content of 40-50% from seed. The oil was found to contain high levels of unsaturated fatty acids, especially oleic and linoleic (Nzikou, *et al*., 2009). However, it is very low fruits, uneven ripening and less number of female flowers. Previous studies on *Jatropha* focused on only medical and chemical compound. Therefore, an understanding of population genetic diversity for breeding program is needed.

Recently, molecular markers are a powerful technique for investigate genetic diversity of plants due to independent of the influence of environment. Moreover, molecular markers provide polymorphism that required to measure genetic relationships and genetic diversity in a reliable manner (Murty *et al*., 2013). ISSR technique was developed by Zietkiewicz *et al*. (1994). ISSRs are semi-arbitrary markers amplified by PCR in the presence of one primer complementary to the target microsatellite-primed PCR. Amplification does not require genome sequence information and lead to multilocus and highly polymorphous patterns. Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSR markers are quick and easy to handle, but

they seem to have the reproducibility of simple sequence repeat (SSR) marker because of the longer length of their primers (Bornet and Branchard, 2001). ISSR method has been used to detect genetic diversity of *Jatropha* from Malaysia, Philippine, India and Indonesia (Biabani *et al*., 2013). Hence, the present study was conducted using ISSR technique to reveal the genetic diversity of *Jatropha* in Thailand.

MATERIALS AND METHODS

Plant Samples

16 accessions of *Jatropha curcas* were collected from Agricultural Research and Developmen in Nakromrachasima and 2 accessions were correct from *Jatropha* farm, Naresuan University. *J. gossypifolia, J. integerrima, J. podagrica, J. multifida and Ricin communis* (outgroup) were collected from many area in Thailand (Table 1)

Table 1: List of Sample Used in this Study

Sample	Accession No	Source	Collection site
J. curcas	J1	Nakronsawan	a
J. curcas	J2	South-Africa	a
J. curcas	J3	Lampoon	a
J. curcas	J4	Khankaen	a
J. curcas	J5	Lampoon	a
J. curcas	J6	India	a
J. curcas	J7	Chiang mai	a
J. curcas	J8	Myanmar	a
J. curcas	J9	Chiang mai	a
J. curcas	J10	Chiang mai	a
J. curcas	J11	Senegal	a
J. curcas	J12	Laos	a
J. curcas	J13	Lampang	a
J. curcas	J14	Mukdahan	a
J. curcas	J15	Chiang mai	a
J. curcas	J16	Loie	a
J. curcas	J17	Phitsanulok	b
J. integerrima	J18	Chiang mai	b
J. integerrima	J19	Phitsanulok	b
J. gossypifolia	J20	Phichit	b
J. curcas non-toxic	J21	Mexico	b
J. curcas non-toxic	J22	Mexico	b
J. podagrica	J23	Nakronrachasima	b
J. multifida	J24	Phitsanulok	b
R. communis	R25	Phitsanulok	b

*[a] Agricultural Research and Development in Nakromrachasima [b] Naresuan University, Phisanulok

DNA Extraction and Inter Simple Sequence Repeat (ISSR) Analysis

Total genomic DNA was extracted from the young leaves of twenty-five samples using CTAB method (Dhakshanamoorthy and Selvaraj, 2009). PCR amplification was performed with thirty-nine ISSR primers. Reactions were carried out in a total volume of 20 µl consisting of 20 ng of template DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 200 nM primers, 1.0 unit of *Taq* polymerase and sterile water. Amplifications were made in a Perkin Elmer 9600 thermocycler with an initial denaturing step of 3 min at 94 °C, followed by 35 cycles of 30 s at 94°C, 45 s at 52°C, 1.30

min at 72°C and a final extension of 5 min at 72°C. PCR products were separated by electrophoresis on 1.6% agarose gels in TBE buffer and visualized using ethidium bromide staining.

DATA ANALYSIS

DNA fragments were scored as presence (1) or absence (0) for each primer. These scores were used to calculate genetic similarity according to Nei and Li (1997), from which a UPGMA cluster dendogram was constructed using NTSYS-pc 2.20e (Rohlf, 2000)

RESULTS AND DISCUSSIONS

Thirty-nine ISSR primers were used for prescreening assay with six samples, only five primers (Table 2) generated clear band pattern and polymorphism were selected for further analysis. A total of 54 polymorphic bands were found across of six ISSR primers. The number of bands amplified by each primer ranged from 8 to 14 with an average of 10.5 and the fragments ranging from 0.35-2.3 kb. The primer based on the poly (GACA) revealed more polymorphisms than the primer based on any other motif. However, almost the selective primers were composed of dinucleotide repeats. According with many reports studied genetic diversity in plants.

ISSR data were calculated using UPGMA and analysis was done by using NTSYS-pc (Version 2.20e). The similarity index revealed that the species fell in the range of 0.66-1.00 among *J. curcas* and interspecific level of *Jatropha* ranged from 0.472- 0.717 (Table 3). Maximum similarity was between *J. curcas* 5 and *J. curcas* 12 (100%), while least similarity were found between *J. curcas* 11 and *J. podagrica*, *J. curcas* 17 and *J. podagrica* (47.2%). The dendrogram (Figure 1) classified the 24 accessions into three clusters. All accessions of *J. curcas* and *J. multifida* were clustered into group I. Two accessions of *J. curcas* non-toxic had a relatively high genetic diversity (0.849), similarity *J. curcas* 7, 9 and 10 (Chiang-mai, Thailand) and *J. curcas* (Myanmar) were placed within the same sub-clade and two groups were also in accordance with geographical origin.

The similarity index suggested *J. curcas* 5 (Lampoon, Thailand) that was closely related to *J. curcas* 12 (Laos) but difference geographical origin. *J. curcas* was closely related to *J. multifida* and they represented the same number and structure of chromosomes (2n=22) (Sasikala and Paramathama, 2010). The second group contained *J. integerrima* and *J. gossypifolia*. However, in contrast to previous studies on genetic diversity of *J. curcas* using AFLP and ISSR markers showed that *J. integerrima* was genetically more similar to *J. curcas* than the others (Soonthornyatara, *et. al*., 2015). Group 3 was *J. podagrica*.

In the present study, the genetic variation of *J. curcas* accessions from Thailand and other countries revealed very low genetic variation among the samples. There were a large number of identical samples. According to Shen *et al*. (2010) represented the use of AFLP marker to evaluate genetic diversity of *J. curcas* in Hainan, China. The result showed a high similarity. In the same way, Pamidimarri *et al.* (2010) used AFLP and RAPD markers to evaluate the genetic diversity of 28 samples of *J. curcas* collected from distinct geographical areas in India and a high similarity ranged from 0.866 to 1.00. *J. curcas* was considered the ancestral primitive species that represented its morphological distinctive characteristics (Dehgan and Webster, 1979).

CONCLUSIONS

Genetic diversity in the genus *Jatropha* from Thailand was used to study by ISSR technique. This study represented that *J. curcas* had low genetic diversity and has to import a new *J. curcas* from other counties or increase the genetic diversity of *J. curcas* by induces mutation using chemical reagent.

Table 2: List of ISSR Polymorphic Primers Sequence, Number of Polymorphic Bands Amplified and Product Size

No.	Primer Code	Primer Sequence(5'- 3')	Total Bands	Size Bands (bp)
1	UBC808	AGA GAG AGA GAG AGA GC	8	400-2000
2	UBC827	ACA CAC ACA CAC ACA CG	9	450-1500
3	UBC835	AGA GAG AGA GAG AGA GYC	12	350-2000
4	UBC873	GAC AGA CAG ACA GAC A	14	400-2000
5	MAO	CTC CTC CTC CTC RC	11	400-2300
		Total	**54**	**350-2300**

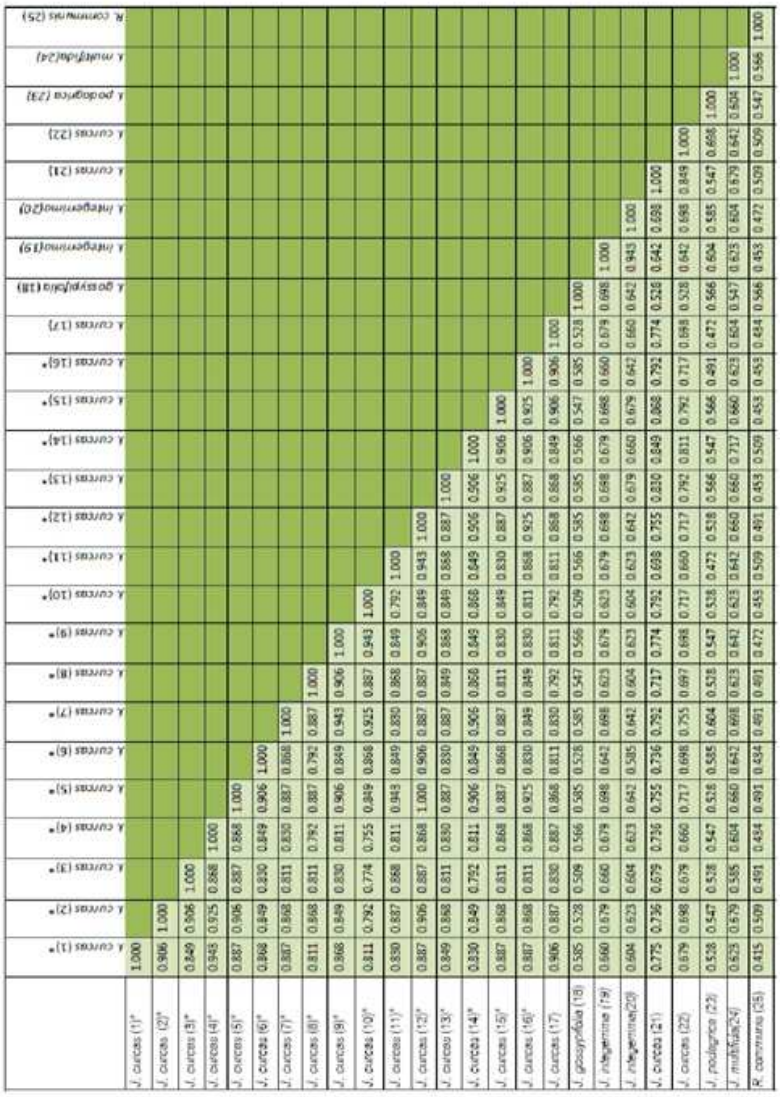

Table 3: Similarity Index of 24 Accessions of *Jatropha*

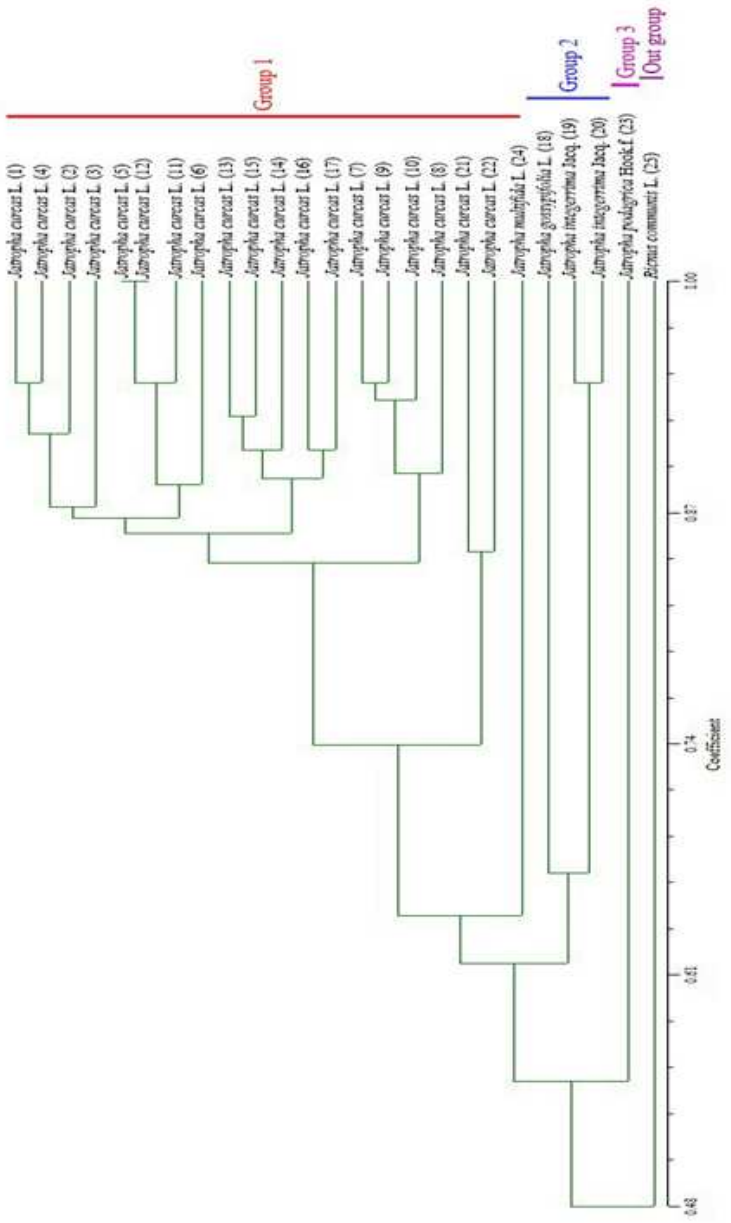

Figure 1: Dendroagram Show the Relationships Among The 24 Accession of Jatropha.the Dendrogram Was Generated from Similarity Index Based on UPGMA

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